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Age-related changes in skin permeability of hydrophilic and lipophilic compounds in rats

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The effect of age on intact and stripped skin permeability of lipophilic (ketoprofen and isosorbide dinitrate) and hydrophilic permeants (deuterium oxide and diclofenac sodium) was investigated using STD: Wistar male rats aged 5 to 180 days. The permeability of permeants through intact skin increased with increasing lipophilicity of the permeants at each age, indicating that the permselective property of rat skin is revealed even at 5-days-old. The permeability coefficients through intact skin decreased with increasing age, and the extent of these decreases was higher for lipophilic permeants than that for hydrophilic permeants. On the other hand, the stripped skin permeability of permeants was almost the same at each age, and with aging each permeability coefficient through stripped skin decreased up to 21 days, dramatically during 21–90 days and then gradually again to 180 days. The thickness of the stratum corneum and stripped skin increased according to age with faster growth during 21–90 days. The reciprocal of the mean thickness of stratum corneum and stripped skin correlated well with intact skin and stripped skin permeability ($r > 0.9$), respectively. These results clarified that the permselectivity of rat skin against lipophilicity of permeant exists at the latest from 5 days after birth. In addition, it is speculated that the thickness of skin is a large factor in the decrease of its permeability with age.

1. Introduction

The skin acts as an effective barrier to the absorption of many substances including drugs [1]. Percutaneous absorption not only depends on the physical and chemical characteristics of the penetrant but also on the characteristic of skin barrier. The skin of aged animals exhibits many structural and functional alterations compared with young animals [2, 3]. It is interesting to study age-related skin permeability since the knowledge can be applied to estimate the potential ramifications for prescribing drugs for different age populations. Skin permeability of a preterm infant is known to be generally higher than that of a full term infant and adult [4, 5]. However, there is no consensus on the difference of skin permeability between young and old adults [6–11].

In the present study, age-related skin permeability was investigated for rats with several penetrants through intact and stripped skin at various ages (5, 10, 21, 90, and 180 days old). Ketoprofen (KP) and isosorbide dinitrate (ISDN) were used as model lipophilic permeants, and deuterium oxide (D_2O) and diclofenac sodium (DC-Na) were used as hydrophilic permeants. The relationship between permeability of penetrants and structural characteristics of the rat skin at various ages was also evaluated.

2. Investigations and results

2.1. Effect of aging on skin permeability of various permeants

Table 1 shows the permeability coefficient (P) of various permeants through intact and stripped skin of rats at various ages. Permeability through intact skin depended on the lipophilicity of permeant at each age, and P was highest in KP (log $K_{ow} = 3.11$) followed by ISDN (log $K_{ow} = 1.34$), D_2O and DC-Na (log $K_{ow} = -0.962$) [12]. Moreover, P of all permeants decreased with increasing age. In stripped skin, P at each age was almost the same among permeants except DC-Na, and a decrease in P with age was also found for all permeants. The degree of decrease in the permeability of each permeant with aging was clarified by normalized P values with those of 5-day-old rats. Normalized permeability coefficients through intact skin (P_{NI}) are shown in Fig. 1. Although P_{NI} decreased with age for all permeants, the degree of decrease was higher for lipophilic permeants than that for hydrophilic permeants. Fig. 2 shows normalized permeability coefficients through stripped skin (P_{NS}). P_{NS} decreased gradually with age to 21 days, dramatically from 21 to 90 days and gradually again to 180 days. Moreover, the degree of decrease in P_{NS} was independent of the lipophilicity of the permeant.

Table 1: Permeability coefficient of permeants through intact and stripped skin of rats at verious ages[a]

P	Age (days)				
	5	10	21	90	180
Intact skin ($\times 10^{-2}$ cm/h)					
KP	7.07 ± 0.93	6.26 ± 0.78	5.94 ± 0.67	4.19 ± 0.71	2.87 ± 0.49
ISDN	3.96 ± 0.51	3.06 ± 0.30	3.24 ± 0.19	2.44 ± 0.32	2.01 ± 0.25
D_2O	0.393 ± 0.041	0.357 ± 0.024	0.354 ± 0.037	0.035 ± 0.017	0.300 ± 0.0037
DC-Na	0.0603 ± 0.0093	0.0571 ± 0.0068	0.0594 ± 0.0097	0.0482 ± 0.0045	0.0453 ± 0.0037
Stripped skin (cm/h)					
KP	0.947 ± 0.200	0.855 ± 0.063	0.855 ± 0.200	0.294 ± 0.058	0.242 ± 0.042
ISDN	0.817 ± 0.173	0.770 ± 0.089	0.783 ± 0.130	0.340 ± 0.038	0.230 ± 0.038
D_2O	0.692 ± 0.104	0.686 ± 0.054	0.646 ± 0.075	0.304 ± 0.089	0.227 ± 0.020
DC-Na	0.254 ± 0.023	0.243 ± 0.034	0.239 ± 0.034	0.072 ± 0.022	0.064 ± 0.011

[a] Each value represents the mean ± S.D. of three to six experiments

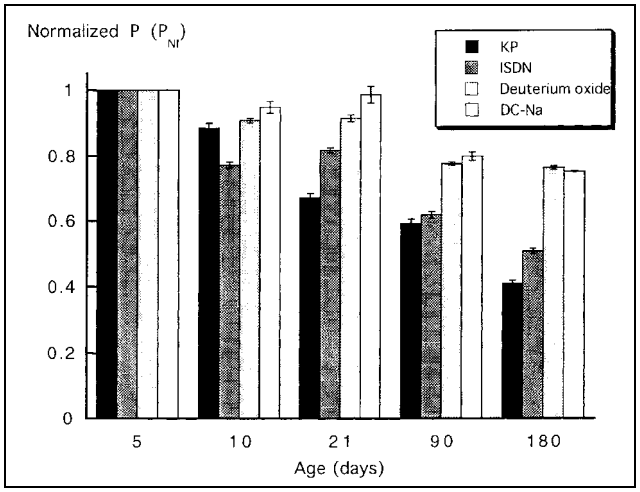

Fig. 1: Normalized permeability coefficient of permeants through intact skin of rats at various ages. Each value is the ratio of the permeability coefficient of each permeant at each age to that at 5 days and represents the mean ± S.D. of three to six experiments

2.2. *Structural characteristics of rat skin at various ages*

To determine the causes for age-related differences in permeability through intact and stripped skin, we focused on the structural characteristics in both membranes. Membrane thickness is one of the main factors affecting the permeability of the penetrant through it. Thickness of the stratum corneum, viable epidermis, dermis, and whole skin of rats was thus measured (Table 2). Thickness of the stratum corneum gradually increased with age. Although the thickness of stripped skin, composed of viable epidermis and dermis, also increased with age, the dermal thickness dramatically increased from 21 to 90 days of age.

Table 2: Thickness of skin strata of rats at various ages[a]

Age (days)	Stratum corneum (μm)	Epidermis (μm)	Dermis (μm)	Whole skin (mm)
5	13.9 ± 3.2	13.6 ± 2.2	243.3 ± 29.8	0.276 ± 0.039
10	16.5 ± 2.4	14.8 ± 2.6	311.3 ± 31.4	0.330 ± 0.046
21	18.1 ± 2.7	15.9 ± 2.5	350.6 ± 42.8	0.359 ± 0.044
90	23.4 ± 2.9	18.9 ± 2.4	720.8 ± 51.9	0.777 ± 0.052
180	27.5 ± 3.8	22.7 ± 3.4	808.8 ± 69.3	0.853 ± 0.077

[a] Each value represents the mean ± S.D. of three to four experiments

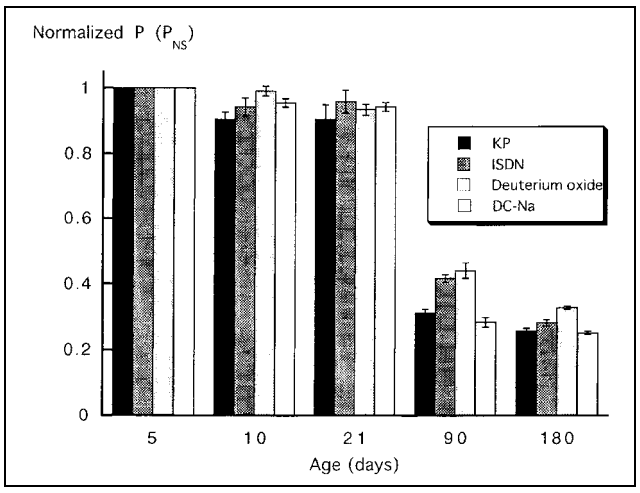

Fig. 2: Normalized permeability coefficient of permeants through stripped skin of rats at various ages. Each value is the ratio of the permeability coefficient of each permeant at each age to that at 5 days and represents the mean ± S.D. of three to six experiments

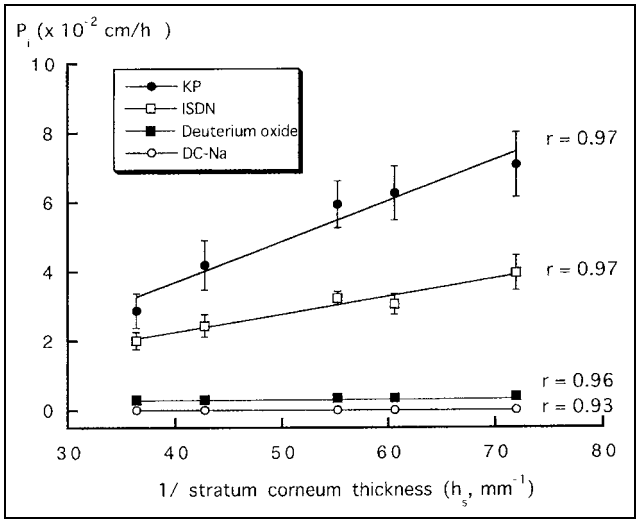

Fig. 3: Relationship between the permeability coefficient through intact skin and mean thickness of the stratum corneum of rats at each age. Each point represents the mean ± S.D. of three to six experiments

2.3. *Relationship between the permeability coefficient and membrane thickness*

For the purpose of clarifying the relation between the skin permeability of permeants and thickness of the skin, P through intact skin (P_i) was plotted against the reciprocal of stratum corneum thickness ($1/h_s$) (Fig. 3). It was evident that P_i correlated well with 1/h ($r > 0.9$). However, the slope for lipophilic drugs was higher than that for hydrophilic drugs (0.12 and 0.053 for KP and ISDN, and 0.0027 and 0.000045 for D_2O and DC-Na). The relationship between P through stripped skin (P_s) and the reciprocal of stripped skin ($1/h_v$) was plotted in Fig. 4. A high correlation coefficient ($r > 0.9$) was obtained, and the slope of each penetrant was almost the same (about 0.2) except for DC-Na (0.081).

3. Discussion

In this study, *in vitro* percutaneous absorption of both hydrophilic (DC-Na and D_2O) and lipophilic permeants

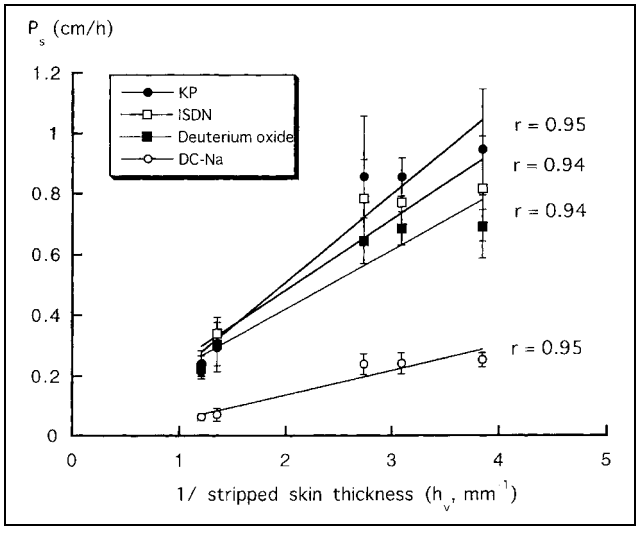

Fig. 4: Relationship between the permeability coefficient through stripped skin and mean thickness of the viable epidermis and dermis of rats at each age. Each point represents the mean ± S.D. of three to six experiments

(ISDN and KP) through intact and stripped skin was evaluated for rats from 5 to 180 days of age. The permeability coefficient through intact skin increased with lipophilicity of the permeants at each age (Table 1). The same phenomenon is generally observed in adult skin of various animals and humans [12–14]. It was confirmed that the permselective property of rat skin is revealed even at 5-days-old. In contrast, the permeability coefficient through stripped skin was almost the same among permeants except for DC-Na at each age (Table 1). One cause for lower permeability of DC-Na should be the larger molecule size of DC-Na (MW = 319; D_2O = 20, ISDN = 236, and KP = 254). The permeability coefficients through stripped skin were markedly higher than those through intact skin (Table 1), suggesting that the main barrier of intact skin is in the stratum corneum during the age range tested in this study.

The permeability coefficients through intact skin decreased with increasing age (Table 1). The same tendency has been found for other permeants [7, 9, 15]. The degree of decrease was higher for lipophilic permeants than that for hydrophilic permeants (Fig. 1). Dick and Scott reported that age did not significantly influence skin permeability in Wistar-derived Alderly Park rats from 10-to 120-days old [10]. It should be noted that only hydrophilic penetrants, water and mannitol were used in their investigation; therefore, the change in skin permeability with age might not have been detected. Aging also alters permeability of stripped skin: the permeability coefficients decreased with age up to 21 days, dramatically during 21–90 days and gradually again up to 180 days (Table 1).

To clarify the causes for age-related changes in permeability through intact skin, structural characteristics of the stratum corneum, the main permeation barrier, have to be investigated. Thickness of the stratum corneum should be an important factor, namely the pathway length [16]. The mean thickness of the stratum corneum showed a trend to increase with age (Table 2). High correlation coefficients between permeabilities of permeant through intact skin (P_i) and the reciprocal of mean thickness of the stratum corneum ($1/h_s$) were obtained. The difference in the slope of P_i versus $1/h_s$ reflected the different partition coefficient of permeants (Fig. 3). These results strongly suggest that thickness plays an important role in the age-related difference in the permeability of rat skin. We have disregarded the transfollicular pathway in this study, which was discussed as a main factor in some reports [6, 10], since it is a very small area fraction in skin surface (about 0.1%) [17].

As causes of the age-related decrease of permeability through stripped skin, we focused on two main factors, water content and thickness of stripped skin (viable epidermis and dermis), which may reflect the porosity and pathway length of the porous membrane. Although a significant difference of water content among these ages was not found (from 59.3 ± 1.3% at 5 days to 57.7 ± 1.4% at 180 days, not shown), the thickness of both the viable epidermis and dermis increased as a function of age (Table 2), similar to data reported by Tregear [18] and Dick and Scott [10]. The dramatic increase in the thickness, especially the dermis thickness between 21 and 90 days (Table 2) was observed in an opposite fashion to permeability through stripped skin (Table 1).

Considerably high correlation coefficients between permeabilities through stripped skin (P_s) and the reciprocal of thickness of the viable epidermis and dermis ($1/h_v$) were obtained (Fig. 4), and the slope, which reflects diffusivity of penetrants was almost the same except for DC-Na. In comparison with the viable epidermis, the dermis is known to offer the bulk of the resistance through stripped skin [19]. The change in permeability across stripped skin with age might come as a consequence of the change in dermis thickness. However, tendency of plateau of P_s, especially between 2.5 and 4 mm^{-1} in $1/h_v$ was also observed in both hydrophilic and lipophilic compounds (Fig. 4). This phenomenon might be discussed with the matter that the tortuosity in the membrane may also affect the skin permeability through the porous route in the skin [12] or with the possibility of altered barrier function itself of the dermis with aging. This problem should be considered in the future study.

The present study confirmed that age affects the permeability of permeants through both intact and stripped skin of rats. The difference in permeability with age was primarily caused by the increase in thickness of skin strata, which should be considered an important factor for the limited percutaneous absorption in all ages.

4. Experimental

4.1. Materials

Ketoprofen (KP) and diclofenac sodium (DC-Na) were purchased from Wako Pure Chemical Industries Co. (Osaka, Japan). Isosorbide dinitrate (ISDN) and the deuterium oxide (D_2O) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA) and Merck Co. (Darmstadt, Germany), respectively. All these drugs and chemicals were used without further purification. Other chemical and solvents were of reagent grade and obtained commercially.

4.2. Skin membrane preparation

Male rats (STD: Wistar strain) at various ages (5, 10, 21, 90, and 180 days) were supplied by Japan SLC Inc. (Hamamatsu, Japan). Under anesthesia induced by pentobarbital (50 mg/kg, i.p.), the abdominal skin of the rat was carefully shaved and quickly excised with trimming the subcutaneous fat and other extraneous tissue. This skin sample was used as an intact skin. Stripped skin was also obtained by stripping the stratum corneum of the shaved abdominal rat skin 20 times with adhesive cellophane tape (48 mm width, 3 M Co., Minnesota, USA) and excising the skin as above mentioned. These both intact and stripped skin samples were immediately used in diffusion experiment, respectively.

4.3. Skin permeation procedure

Either intact or stripped skin was mounted between two half cells of a side-by-side diffusion cell with a water jacket connected to a water bath at 37° C, each having a 3.0 ml volume and 0.95 cm^2 effective diffusion area [20]. The receiver and donor compartments were filled with distilled water and stirred with a Teflon magnetic stirrer at 1440 rpm. One hour of equilibration was allowed, and then the receiver compartment was filled with freshly distilled water for D_2O and DC-Na or 20% w/w polyethylene glycol 400 for KP and ISDN to maintain a sinking condition in the receiver solution. The donor compartment was replaced with D_2O or a drug suspension in distilled water (at 2–10 times higher concentration than the solubility of each drug) to assure constant thermodynamic activity throughout the course of the experiment. A part (0.5 ml) of the receiver solution was withdrawn every hour for 8 h and replaced with the same volume of distilled water to keep the volume constant. The concentration of permeants in the samples was analyzed, and the cumulative amount was plotted against time. The permeability coefficient (P) of permeants was determined by dividing the slope of the steady-state portion (3–8 h) of these plots by the solubility of permeants at 37° C. Metabolites were not detected in either the donor or the receiver solution throughout the experiment.

4.4. Analytical methods

D_2O was quantified by measuring the intensity of the O-D stretching vibration band at 2512 cm^{-1} in infrared spectroscopic spectra [21]. Briefly, the absorbance of the D_2O sample in a calcium fluoride cell (JASCO corporation, Tokyo, Japan), with a thickness of 0.057 mm was determined with Fourier Transform Infrared Spectrophotometry (FT/IR-230, JASCO corporation, Tokyo). KP, DC-Na and ISDN were assayed by HPLC. The HPLC system consisted of a pump (LC-6AD, Shimadzu, Kyoto, Japan), a sperisorb ODS 5-C18, 4.6-mm × 250 mm column (YMC, Co., LTD, Kyoto), an auto-injector (SIL-9A, Shimadzu), a variable UV detector

(SPD-10A, Shimadzu) and an integrator (C-R5A, Shimadzu). In all cases, the flow rate of the mobile phase was 1.0 ml/min and the column temperature was 40° C. The mobile phase of methanol and 0.1% phosphoric acid (75 : 25) was used for KP and DC-Na, and acetonitrile and water (55 : 45) were for ISDN. The internal standards were p-hydroxy benzoic acid amyl ester, butyl ester and ethyl esters, and the detector wavelengths were set at 254, 286, and 220 nm for KP, DC-Na and ISDN, respectively.

4.5. Measurement of skin thickness

The thickness of the stratum corneum, viable epidermis, dermis and whole skin of rat skin was determined by the method of Evans and Rutter [22] with a slight modification. The skin specimens were fixed in 3% formaldehyde in 0.1 M phosphate buffer pH 7.2 and were dipped in paraffin. The sections were cut and stained with hematoxylin-eosin and then observed under lightmicroscopy (BHS-324, Olympus, Tokyo).

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